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The Morquio A Syndrome (Mucopolysaccharidosis IVA) Gene Maps to 16q24.3

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Summary

The gene for N-acetylgalactosamine-6-sulfatase, the deficiency of which results in Morquio A syndrome (mucopolysaccharidosis type IVA), was assigned to chromosome 16 at band q24.3 by fluorescence in situ hybridization. Localization to this band was confirmed by PCR analysis of a somatic cell hybrid panel used for fine mapping of chromosome 16.

Introduction

N-acetylgalactosamine-6-sulfatase (GALNS; E.C.3.1.-6.4; gene symbol, *GALNS*) is one of a series of lysosomal enzymes involved in the degradation of the glycosaminoglycans, keratan sulfate, and chondroitin-6-sulfate (see Roden 1980). Deficiency of GALNS in humans results in the autosomal recessive disorder Morquio A syndrome, otherwise known as mucopolysaccharidosis (MPS) type IVA (see Neufeld and Muenzer 1989).

Recently Tomatsu et al. (1991) reported the isolation and expression of a cDNA clone encoding GALNS. We have used this sequence to isolate a full-length *GALNS* cDNA clone from a human endothelial library (X.-H. Guo, J. J. Hopwood, and C. P. Morris, unpublished data). In this communication, we report the use of this clone and the published sequence of *GALNS* to localize *GALNS* by in situ hybridization to human metaphase chromosomes and by PCR amplification from a set of cell hybrids containing portions of human chromosome 16q (Callen et al. 1992).

Material and Methods

Probe

We have used the published sequence of *GALNS* (Tomatsu et al. 1991) to generate a 140-bp PCR product from the 5′ end of human fibroblast *GALNS* mRNA. This was used as a probe to isolate a full-length cDNA clone of 2.3 kb from a human endothelial cDNA library, which has been sequenced over half its length and found to extend from nucleotide position 26 to nucleotide 2328 of the published sequence (X.-H. Guo, J. J. Hopwood, and C. P. Morris, unpublished data).

Cell Lines

Construction and characterization of the mouse-human (CY) hybrid cell lines has been described by Callen et al. (1992). Their chromosome 16 content is shown in table 1.

PCR Amplification of Hybrid Cell Lines

The primers G6S8 (5′-CCACTAGCACCTGCGCAGAC-3′) and G6S9 (5′-GGCAGGGTCCTGAGGTCTGA-3′) were used to amplify a region of 266 bp from the most 3′ exon of the *GALNS* gene, between nucleotide positions 1618 and 1883, according to the numbering of Tomatsu et al. (1991). Amplification was carried out using 250 ng of genomic DNA, 200 ng of each primer, and 2 units of *Taq* polymerase (Cetus) in the recommended buffer, except that the reactions contained 400 μM dNTPs, 2.5 mM $MgCl_2$, 0.1% (v/v) Triton X-100, and 10% (v/v) dimethylsulfoxide. After 7 min at 96°C, the reactions were subjected to 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 45 s. This PCR product was from the 3′ untranslated region of the gene, and the primers did not cross-react with the mouse DNA in the hybrids.

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Table 1

Assignment of *GALNS* by PCR Analysis of Hybrid Lines

Cell Line	Chromosome 16 Content	Hybridization
CY18	Complete 16	+
CY3	pter→q24.2	–
CY2	q24.2→qter	+
CY100	q24.1→qter	+
CY120	q24.1→qter	+
CY115	q24.1→qter	+
CY18A	q24.2→q24.3	+

In Situ Hybridization

The *GALNS* cDNA probe was nick-translated with biotin-14-dATP and hybridized in situ at a probe concentration of 15 ng/μl to metaphases from two normal males. The method was modified from that described elsewhere (Callen et al. 1990), in that chromosomes were stained before analysis with both propidium iodide (as counterstain) and DAPI (for chromosome identification).

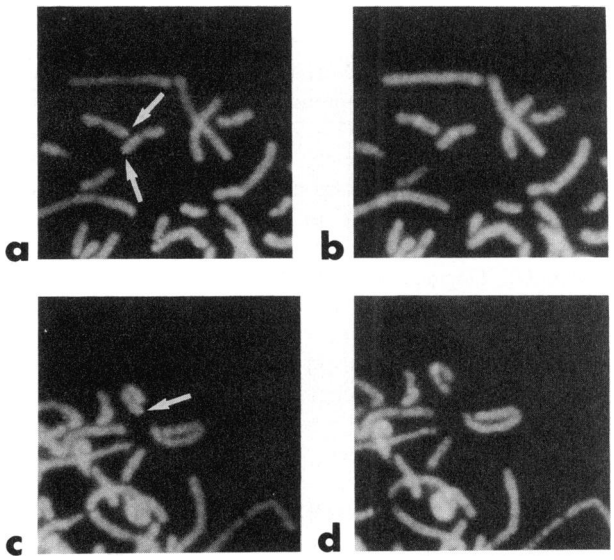

Figure 1 Partial metaphases showing fluorescence in situ hybridization of the biotinylated *GALNS* probe. *a* and *c*, Normal male chromosomes stained with propidium iodide. Hybridization sites on chromosome 16 are indicated by arrows. *b* and *d*, DAPI staining of the same metaphases, for chromosome identification.

Results

After in situ hybridization with the *GALNS* probe, 30 normal male metaphases were examined for fluorescent signal. Twenty-five of these metaphases showed signal on at least one chromatid of chromosome 16, in the distal region of 16q at band q24.3 (fig. 1). There were a total of 13 nonspecific background dots observed in these 30 metaphases. A similar result was obtained from the hybridization of *GALNS* to the second normal male (data not shown).

PCR analysis of the hybrid cell panel showed (table 1) that the human leukocyte DNA and hybrid cell lines CY18, CY2, CY100, CY120, CY115, and CY18A contained the *GALNS* sequence, as shown by the presence of specific products of 266 bp. These bands were absent in the nonhuman DNA controls and from the hybrid cell line CY3, and no nonspecific bands were present. The chromosome 16 material unique to the lines containing the *GALNS* sequence is 16q24.2→16q24.3, which is consistent with the localization from the in situ hybridization result.

Discussion

The MPS are a group of lysosomal storage disorders caused by a deficiency of individual enzymes responsible for the degradation of mucopolysaccharides. We have localized N-acetylgalactosamine-6-sulfatase (MPS type IVA) to 16q24.3. α-L-iduronidase (MPS-I) is on 4p16.3 (Scott et al. 1990); iduronate-2-sulfatase (MPS-II) is on Xq28 (Wilson et al. 1991); glucosamine-6-sulfatase (MPS-IIID) is on 12q14 (Robertson et al. 1988); β-D-galactosidase (MPS-IVB) is on chromosome 3 (Shows et al. 1979); N-acetylgalactosamine-4-sulfatase (MPS-VI) is on 5q13.3 (Litjens et al. 1989); and β-D-glucuronidase (MPS-VII) is on 7q21.1-q22 (Allanson et al. 1988).

Band q24.3 of chromosome 16 is a region of the genome for which there are currently only two mapped genes (Reeders et al. 1991)—renal dipeptidase 1 (*DPEP1*), a membrane-bound enzyme that hydrolyzes several dipeptides as well as the β-lactam ring of antibiotics such as penem and carbapenem, and *APRT*, which encodes an enzyme in the nucleotide salvage pathway.

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References

Allanson JE, Gemmill RM, Hecht BK, Johnsen S, Wenger DA (1988) Deletion mapping of the β-glucuronidase gene. Am J Med Genet 29:517–522

Callen DF, Baker E, Eyre HJ, Chernos JE, Bell JA, Sutherland GR (1990) Reassessment of two apparent deletions of chromosome 16p to an ins(11;16) and a t(1;16) by chromosome painting. Ann Genet 33:219–221

Callen DF, Doggett NA, Stallings RL, Whitmore SA, Lane SA, Nancarrow JK, Chen LZ, et al (1992) High resolution cytogenetic-based physical map of human chromosome 16. Genomics 13:1178–1185

Litjens T, Baker EG, Beckmann K, Morris CP, Hopwood JJ, Callen DF (1989) Chromosomal localization of *ARSB*, the gene for human *N*-acetyl-galactosamine-4-sulphatase. Hum Genet 82:67–68

Neufeld EF, Muenzer J (1989) The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 1565–1587

Reeders ST, Hildebrand CE, Sutherland GR (1991) Report of the Committee on the Genetic Constitution of Chromosome 16. Human Gene Mapping 11. Cytogenet Cell Genet 58:643–685

Robertson DA, Callen DF, Baker EG, Morris CP, Hopwood JJ (1988) Chromosomal location of the gene for human glucosamine-6-sulphatase to 12q14. Hum Genet 79:175–178

Roden L (1980) Structure and metabolism of connective tissue proteoglycans. In: Lennarz WJ (ed) The biochemistry of glycoproteins and proteoglycans. Plenum, New York, pp 267–371

Scott HS, Ashton LJ, Erye HJ, Baker E, Brooks DA, Callen DF, Sutherland GR, et al (1990) Chromosomal localization of the human α-L-iduronidase gene (*IDUA*) to 4p16.3. Am J Hum Genet 47:802–807

Shows TB, Scrafford-Wolff LR, Brown JA, Meisler M (1979) G_{M1}-gangliosidosis: chromosome 3 assignment of a β-galactosidase-A gene (β-GalA). Somat Cell Genet 5: 147–158

Tomatsu S, Fukuda S, Masue M, Sukegawa K, Fukao T, Yamagishi A, Hori T, et al (1991) Morquio disease: isolation, characterization and expression of full-length cDNA for human *N*-acetylgalactosamine-6-sulfate sulfatase. Biochem Biophys Res Commun 181:677–683

Wilson PJ, Suthers GK, Callen DF, Baker E, Nelson PV, Cooper A, Wraith JE, et al (1991) Frequent deletions at Xq28 indicate genetic heterogeneity in Hunter syndrome. Hum Genet 86:505–508